Glycan Derivatives That Possess Chaperone-Like Activity

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Proteins are unstable at high temperatures and under acidic/basic conditions, and they are easily denatured and inactivated even at room temperature. The difficulty in handling such proteins is one of the obstacles for protein research. Under these circumstances, many small molecules have been reported that prevent the aggregation of proteins [Shiraki (2004) *Seibutsu* 87–90]. Recently, glycan chains and glycan derivatives have been also reported that have effects that prevent protein aggregation and facilitate protein folding. Here, we would like to introduce some glycan derivatives that possess chaperone-like activity.

It is well known that calnexin and calreticulin, the endoplasmic reticulum (ER)-localized lectins, recognize monoglucosylated *N*-glycans (a) on nascent glycoproteins and prevent their aggregation and increase their folding efficiency [Helenius *et al.* (1994) *Mol. Biol. Cell.* 253–265]. In addition to this indirect contribution to protein folding, Yamaguchi *et al.* pointed out the direct contribution of *N*-glycans to the folding of proteins [Yamaguchi *et al.* (1996) *J. Biochem.* 474–477]. They reported that not only high-mannose-type and complex-type *N*-glycans bind to proteins, but free *N*-glycans also facilitate the refolding of various denatured proteins, such as RNase A, egg white lysozyme, and bovine α -lactalbumin [Kimura *et al.* (1998) *J. Biochem.* 857–862]. X-ray crystal structure and NMR analyses demonstrated that the *N*-acetylglucosamine (GlcNAc) residue of *N*-glycan forms a hydrophobic surface and interacts with exposed hydrophobic amino acid residues to protect the protein surface from the hydrophilic environment [Sutton *et al.* (1983) *Biochem. Soc. Trans.* 130–132; Erbel *et al.* (2000) *Biochemistry* 6012–6021]. Recently, similar effects were examined for free *N*-glycans in plants [Tanaka *et al.* (2015) *Glycoconj. J.* 193–194].

In addition, sulfated disaccharide [D2S0, (b)], derived from heparin, has a chaperone-like effect on mutated 2-*O*-sulfurized iduronate-2-sulfatase (IDS), which causes mucopolysaccharidosis type II. It was reported that D2S0 improves the folding, stability, and activity of the enzyme [Hoshina *et al.* (2018) *Mol. Genet. Metab.* 118–122; Lawrence *et al.* (2008) *Nat. Methods.* 291–292]. Mucopolysaccharidosis type II develops as a result of a genetic mutation in IDS, which decreases the amount of enzymes that retain the active structure and leads to the accumulation of glycosaminoglycans in the lysosome. When D2S0 was incubated with recombinant human IDS, the disaccharide attenuated the thermal degeneration of the enzyme in a dose-dependent manner. D2S0

also improved the activity of mutant IDS in patient fibroblasts. Furthermore, D2S0 increased the enzymatic activity of IDS mutants derived from 6 of 7 different mutations in HEK293T cells. These results suggest that D2S0 would be a potent pharmacological chaperone for mucopolysaccharidosis type II. As D2S0 inhibits the enzymatic activity of mutant IDS dose-dependently at the optimal temperature, it seems to bind to the active site of mutant IDS to enhance its stability against heat and to improve the folding efficiency, which leads to the maintenance of the enzymatic activity.

Chaperone-like activities of polysaccharides have been reported as well. Rezaii and Khodagholi reported that a polysaccharide alginate (c) contained in seaweed has chaperone-like activity, which enhances the thermal stability of proteins and assists with refolding [Rezaii *et al.* (2009) *Protein J.* 124–130]. The alginate chain consists of two uronic acids (β -D-mannuronic acid (M) and α -L-guluronic acid (G)), which are 1,4 linked in a pyranose form and linearly polymerized. The composition ratio of M and G is not uniform and depends on the type and site of seaweed. It is known that the addition of copper, calcium, or zinc ions converts alginate into a gel form. A comparison of the chaperone activity using alkaline phosphatase (ALP) as a model protein showed that ALP increased the stability to a higher degree in the presence of the alginate gel beads; whereas, denatured ALP was refolded with a higher yield in an aqueous sodium alginate solution.

The functionalization of artificial polysaccharides has been studied. Akiyoshi *et al.* found that the nanoparticles formed spontaneously by hydrophobized polysaccharides have chaperone-like activity [Akiyoshi *et al.* (1996) *Supramol. Sci.* 157–163; Akiyoshi *et al.* (1999) *Bioconjugate Chem.* 321–324]. They discovered that hydrophobized polysaccharide molecules, in which cholesterol groups were partially introduced into water-soluble pullulan (d), self-aggregated in aqueous solution to form stable nanoparticles with a diameter of several tens of nanometers. The cholesterol substituted pullulan (CHP) contained about 5% hydrophobic groups out of the whole molecule and more than 80% water even after nanoparticle formation. Thus, it formed a nanogel by crosslinking hydrophobic groups. The greatest feature of this nanogel is that its dynamic structure can incorporate proteins and peptides by hydrophobic interactions. For example, heat-denatured carbonic anhydrase B (CAB) was complexed with the nanogel and was completely prevented from irreversible aggregation. After the addition of cyclodextrin, the cholesterol crosslinking point was taken into

cyclodextrin, and the self-aggregation of the CHP was dissociated to result in the release of CAB. It was revealed that the released CAB refolded to its native form and almost completely recovered its activity. Recently, the nanogel has been applied to a drug carrier and has attracted much attention in DDS research [Sawada *et al.* (2017) *J. Biomater. Sci. Polym. Ed.* 1183–1198].

Membrane proteins that possess hydrophobic domains immediately aggregate in aqueous solution, and thus, it is difficult for them to retain/form active structures *in vitro*. Recently, a glycolipid [MPlase, (e)] has also been reported that acts like a chaperone [Nishiyama and Shimamoto *et al.* (2012) *Nat. Commun.* 1260]. The glycolipid MPlase was discovered as an inner membrane component, essential for membrane protein integration in *Escherichia coli*. MPlase has a glycan that consists of three kinds of amino sugars [GlcNAc, *N*-acetylmannosamine uronic acid (ManNAcA), and *N*-acetyl-4-aminofucose (Fuc4NAc)], and the trisaccharide unit repeats about ten times. The glycan is linked to diacylglycerol through a pyrophosphate linker. Besides, about 30% of the 6-hydroxyl groups in GlcNAc are acetylated. Experiments using gel filtration chromatography have demonstrated that the phosphorylated glycan of MPlase prevents the aggregation of the substrate protein (pf3 phage coat protein) in aqueous solution. According to a structure–activity relationship study, the long glycan chain (trisaccharide unit $\times 10$), the 6-OAc group of GlcNAc, and the phosphate group contribute to the prevention of protein aggregation in aqueous solution, and it was verified that the chaperone-like

activity of the glycan is one of the factors that increases membrane protein integration efficiency [Fujikawa *et al.* (2018) *ACS Chem. Biol.* 2719–2727]. MPlase is anchored in the inner membrane of *E. coli* by a diacylglycerol moiety, and the phosphorylated glycan that is oriented on the cytoplasmic side seems to capture a membrane protein that is synthesized by the ribosome in the cytoplasm, prevent its aggregation, and integrate it into the inner membrane.

As we have already mentioned, hydrophobic proteins are difficult to handle, because they can be easily denatured, can aggregate, and can be deactivated depending on the temperature, solvent environment, and storage conditions. Under these circumstances, glycan derivatives that possess chaperone-like activities have been reported. Looking at these chaperone-like molecules, the following structures can be considered as characteristic: polar functional groups, such as an amino group, a carboxyl group, a phosphate group, or a sulfate group; hydrophobic functional groups, such as a cholesterol group, an acetyl group, a methyl group, or an olefin; and an acetamide group, which can be a hydrogen bond acceptor and donor. Although the mechanisms by which these chaperone-like molecules prevent protein aggregation differ from each other and still remain unclear, the molecules seem to be interacting with the proteins, catalyzing redox reactions of the proteins, increasing the protein solubility, charging the protein, and dissociating proteins from each other by electrical repulsion. The elucidation of the prevention mechanism of protein aggregation is essential for the development of more effective anti-aggregation (solubilizing)

Fig. 1. Glycan derivatives that possess chaperone-like activity.

agents and pharmacological chaperones and is also important for protein research and the development of therapeutic methods for various diseases related to the misfolding of protein structures.

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